Journal of Proteomics 196 (2019) 81–91

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Comparative proteomics and metabolomics of JAZ7-mediated drought tolerance in *Arabidopsis*

Li Meng[a,b], Tong Zhang[b], Sisi Geng[b], Peter B. Scott[b], Haiying Li[a], Sixue Chen[a,b,c,*]

[a] Key Laboratory of Molecular Biology of Heilongjiang Province, College of Life Science, Heilongjiang University, Harbin 150080, China
[b] Department of Biology, Genetics Institute, Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL, USA
[c] Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, FL, USA

ARTICLE INFO

Keywords:
JAZ7
Drought
Quantitative proteomics
Metabolites
Molecular networks
Arabidopsis thaliana

ABSTRACT

Jasmonates (JAs) are important phytohormones that regulate a wide range of plant processes, including growth, development and stress responses. Jasmonate ZIM-domain (JAZ) proteins are transcriptional repressors in JA signaling. Overexpression of *JAZ7* was found to confer drought tolerance in *Arabidopsis thaliana* (*A. thaliana*), but the molecular mechanisms are not known. Using Tandem Mass Tag (TMT) quantitative proteomics and targeted metabolomics approaches, we found that 394 unique proteins and 96 metabolites were differentially expressed under drought and/or among the three genotypes (wild type (WT), *JAZ7* knock out (KO) and *JAZ7* overexpression (OE)). Unique and differential proteins/metabolites after each comparison were analyzed to gauge their potential functions in drought tolerance. The proteins and metabolites are enriched in JA and abscisic acid (ABA) signaling pathways, response to stress, photosynthesis, redox and metabolic process.

Biological significance: Drought stress is a global challenge that affects agricultural production. *JAZ7* overexpression led to drought tolerance in *A. thaliana* through modulating photosynthesis, redox, and amino acids, phytohormones and defense metabolites. The results have provided important insights into the JAZ7 regulated molecular networks of drought tolerance. The knowledge may facilitate effort to enhance crop drought tolerance in the era of climate change.

1. Introduction

Drought is a most widespread abiotic stress in agriculture, leading to inhibition of plant growth and development, and yield reduction. For example, drought has caused a > 12% decrease in corn production [1]. With the global warming and climate change, drought is expected to become more frequent and severe [2]. Thus, understanding the mechanisms of plant drought response and tolerance is extremely important for improving plant drought tolerance, agriculture productivity, and global food security.

Plants have evolved a variety of ways to deal with drought stress, e.g., by accelerating their life cycles with early flowering, reducing water loss, and increasing water uptake through morphological modifications. Upon drought, phytohormones including abscisic acid (ABA) and jasmonate acid (JA) have been shown to be involved in regulating the stress responses [3,4]. For example, drought promotes the biosynthesis of ABA, which activates specific transcription factors (TFs) to turn on downstream drought responsive genes. Another consequence of drought is increased production of reactive oxygen species (ROS), which may perturb the cellular redox homeostasis and trigger ROS signaling [5,6]. Plants also actively regulate the abundance and activity of key proteins to cope with drought stress. For instance, the efficiency of RuBisCO is decreased due to the slowing down of photosynthesis under drought [7,8]. In contrast, enzymes in redox regulation such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR) and peroxidase (POD) showed increased abundance/activity to maintain the cellular redox balance [9–13]. In addition, accumulation of certain metabolites such as glutathione, betaine, proline, raffinose and galactinol enhances plant drought tolerance [14]. Thus, plants regulate an integrated molecular network at multiple levels to bring about responses such as stomatal closure to prevent water loss and increase survivability. However, stomatal closure decreases CO_2 intake and net photosynthesis, leading to reduced growth and yield [15]. Understanding the molecular

Abbreviations: JA, jasmonate; *JAZ*, jasmonate ZIM domain; TMT, tandem mass tags; COR, coronatine; SA, salicylic acid; ABA, abscisic acid; KO, knockout; WT, wild type; OE, overexpression

* Corresponding author at: Department of Biology, University of Florida, Gainesville, FL, USA.
E-mail address: schen@ufl.edu (S. Chen).

https://doi.org/10.1016/j.jprot.2019.02.001
Received 27 December 2018; Received in revised form 29 January 2019; Accepted 1 February 2019
Available online 05 February 2019
1874-3919/

mechanisms underlying how plants strike a balance between stress tolerance and growth is a key area of agricultural sciences.

JAs regulate multiple aspects of plant growth, development and stress responses [14,16–19]. Under bacterial infection, JA and its methyl ester increased rapidly, leading to accumulation of secondary metabolites [20]. As key regulators of JA signal transduction, there are 12 jasmonate ZIM-domain (JAZ) proteins in *A. thaliana*. JAZ1 was first shown to repress transcription of JA responsive genes [21]. In the presence of JA (especially the bioactive form JA-isoleucine conjugate (JA-Ile)), JA-Ile binds to its receptor, an F-box protein coronatine insensitive 1 (COI1) to form a E3 ubiquitin-ligase complex with the Skp1/Cullin/F-box (SCF). The JA-SCFCOI1 complex subsequently recruits the JAZ repressors for 26S proteasome degradation [21]. Upon the degradation of the JAZ proteins, multiple TFs (e.g., the basic helix−loop−helix MYC TFs) are relieved from JAZ-mediated repression and activate their respective downstream gene expression [21,22]. The JA-SCFCOI1-dependent proteasome system was shown to degrade several JAZ proteins, including JAZ1, JAZ2, JAZ3, JAZ6, JAZ9 and JAZ10 [23–25]. Different JAZ structures and modes of action may account for the functional diversity of the 12 JAZs [26,27]. However, JAZs and JA pathway was rarely studied in plant drought response. JAZ7 was reported to negatively regulate plant responses to dark-induced leaf senescence and pathogen defense [28–30]. Overexpression of *JAZ7* conferred drought tolerance in Arabidopsis [31], but how JAZ7 plays a role in plant drought response has not been reported.

To discover the functions of JAZ7 in drought stress regulation, drought stress responses of three Arabidopsis genotypes, a *JAZ7* knockout (KO), a *JAZ7* overexpression (OE) and wild type (WT) were compared at morphological, physiological and molecular levels. The OE plants showed much higher drought tolerance and lower stress responses than the WT and KO plants. Tandem mass tags (TMT)-based quantitative proteomics and targeted metabolomics revealed differential proteins and metabolites under drought stress in the three genotypes. The differential proteins and metabolites are enriched in JA and ABA signaling, stress response, photosynthesis and metabolic process. This study highlights the JAZ7 regulated molecular networks and potential pathway crosstalks in plants under drought stress.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of *A. thaliana JAZ7* overexpression mutant (OE, SALK040835C), *JAZ7* knockout mutant (KO, WiscDsLox7H11) and the WT (Col-0) were obtained from the Arabidopsis Biological Resource Center (Columbus, OH, USA). The seeds were sown in a Metromix MVP soil (Sungro Horticulture, Agawam, MA, USA) directly and kept in cold room for 2 days, followed by germination and growth in an environmental growth chamber with a circadian cycle of 8 h light (140 μmol m^{-2} s^{-1}) at 22 °C and 16 h darkness at 18 °C. The seedlings were watered twice a week. The genotype of OE was confirmed as previously described [31], and the homozygous KO was confirmed using PCR with combinations of a T-DNA primer (5′-GACAACTTAAT AACACATTGCGGACGTT-3′) and gene specific primers (5′-CATCATCA AAAACTGCGACAAGCC-3′ and 5′-GGTAACGGTGGTAAGGGG AAGT-3′). Plants of 23-day-old were subjected to drought treatment by withholding water for up to 24 days. The control plants were watered twice a week. Three biological replicates were conducted for all the experiments, except the soil content assay with 10 replicates. For physiological and metabolite assays, leaves from each individual plant were pooled as a biological replicate. For proteomics experiments, each biological replicate contained leaves pooled from three different plants.

2.2. Plant relative water content and soil water content analysis

To determine the plant water status, shoots were taken and weighed for fresh weight (FW), dry weight (DW) and turgid weight (TW). FW was determined immediately after sampling. DW was obtained after desiccation at 80 °C until constant weight. Turgid weight was analyzed after a period of 24 h water saturation in the dark at 4 °C. Plant relative water content (RWC) was calculated as follows: RWC = (FW − DW) / (TW − DW) × 100% [32]. The analysis was repeated three times. To measure soil water loss, the water content of soil was calculated as follows: Water $Content_{soil}$ = (W_{soil} − D_{soil})/D_{soil} × 100%, where W_{soil} is wet weight of pot soil, and D_{soil} is the dry weight of the soil after desiccation at 80 °C until constant weight was reached.

2.3. Chlorophyll content and anthocyanin content

Chlorophyll was extracted from leaves in 80% acetone as previously described [33]. After centrifugation at 20,000*g* for 20 min, the absorbance of the supernatant was measured using a spectrophotometer at 440 nm, 663 nm and 645 nm. Total chlorophyll as well as chlorophyll *a* and *b* concentrations were calculated as previously described [34].

For anthocyanin assay, leaf samples of 0.15 g FW were extracted in 2 ml 80% methanol containing 0.1% HCl at room temperature on an orbital shaker at 200 rpm for 2 h. The extract was centrifuged at 3000*g* for 20 min and the supernatant was transferred into a 15 ml vial. The pellet was re-extracted under the identical conditions, and the supernatant was combined. Total anthocyanins content was measured using a spectrophotometric pH-differential method [35]. Briefly, 0.5 ml of the extract was mixed thoroughly with 3.5 ml of 25 mM potassium chloride, pH 1. The mixture was mixed and allowed to stand for 15 min. The absorbance was then measured at 515 nm and 700 nm. The extract was then combined with 25 mM sodium acetate pH 4.5, and the absorbance was measured at the same wavelength. The total anthocyanin content was calculated using the following equation: Anthocyanin (mg/L) = A × MW × DF × 10^3/(ε × L), where A is difference in absorbance as calculated by A = (A_{520nm} - A_{700nm}) pH 1.0 − (A_{520nm} - A_{700nm}) pH 4.5, MW is the molecular weight of anthocyanin (433.2 g/mol), DF is the dilution factor, ε is the extinction coefficient (29,600 mol^{-1} cm^{-1}).

2.4. Protein extraction, digestion and TMT labeling

Leaf proteins from three biological replicates were extracted using a phenol method and quantified using a EZQ kit from Thermo Scientific (Eugen, OR, USA) as described previously [36]. For each sample, 100 μg total protein was reduced by 10 mM tris(2-carboxyethyl)phosphine (TCEP) at 37 °C for 1 h, and then alkylated by 20 mM iodoacetamide (IAM) at room temperature in the dark for 30 min. The samples were digested with trypsin (Promega, Fitchburg, WI, USA) at an enzyme/substrate ratio of 1:80 at 37 °C for 14 h. The peptides were cleaned up using C18 reverse phase columns (The Nest Group, Southborough, MA, USA), and then lyophilized using a speedvac concentrator (Centrivac, Labconco Inc., Kansas City, MO, USA). TMT 10-plex labeling of the peptides was conducted as previously described [30]. Please refer to Supplemental Fig. S1 for the workflow and design.

2.5. Strong cation exchange (SCX) and liquid chromatography mass spectrometry (LC-MS)

The TMT labeled peptides were fractionated using SCX according to a previous method [36,37]. For each SCX fraction, LC-MS was performed on an EASY-nLC 1000 (Thermo Scientific, San Jose, CA, USA) coupled with an Orbitrap Fusion Tribrid (Thermo Scientific, San Jose, CA, USA). Peptides were loaded on to an Acclaim PepMap100 C18 precolumn (2 cm in length, 75 μm inner diameter, 100 Å pore size and 3 μm particle size), and then separated using an Acclaim® PepMap analytical column (25 cm in length, 75 μm inner diameter, 100 Å pore size and 2 μm particle size). The solvent A was 0.1% formic acid in water and solvent B was 0.1% formic acid and 99.9% acetonitrile. The

flow rate was 300 nl/min, and the following gradient parameters were used: 2 5% of B over 0–5 min; 5–35% of B over 5–40 min, 35–60% of B over 40–45 min, 60–95% of B over 45–46 min and isocratic at 95% of B over 46–60 min. A nanoelectrospray ion source (Thermo Scientific, San Jose, CA, USA) in positive mode was used with spray voltage of 1.8 kV and 285 °C ion tube transfer temperature. A top-speed data acquisition method was used to do full MS, MS2 and MS3 within a cycle of 3 s. The MS1 parameters were: mass range 400–1800 *m/z*, resolution 120,000, automatic gain control (AGC) 2×10^5, maximum injection time (MIT) 50 ms, radiofrequency (RF) lens 60. Peptides with 2–6 charges were isolated using a 1.3 *m/z* isolation window and fragmented by collison induced dissociation (CID). The MS2 parameters included datatype centroid, AGC 1×10^4, and MIT 50 ms. The ion trap isolation was 2 *m/z*, and the MS2 ions were further fragmented by high energy collision dissociation (HCD) to generate MS3 ions including the TMT reporter ions. The MS3 parameters were: 65% normalized collision energy, mass range, 100–500 *m/z*, resolution 60,000, AGC 1×10^5, MIT 120 ms, and RF lens 60. A dynamic exclusion of 36 s was set to prevent repeated sampling of high abundance peptides.

2.6. Proteomics data analysis

The MS data were processed as previously described [30]. Briefly, the raw MS data were searched against the *A. thaliana* Tair 10 database with 32,785 entries (October 26, 2017) using the Proteome Discoverer software (version 2.1.1.21) with the SEQUEST^{HT} algorithm. Proteins were identified with at least two peptides. The peptides with false discovery rate (FDR) < 1% were used for further analysis. A universal control pooled from all the samples (quantified by TMT 131) was used as a reference for data normalization. Reproducibility among different replicates was evaluated by Pearson correlation test in R (version 3.3.1). Proteins with at least two quantification values in three replicates were filtered for statistical analysis (Student's *t*-test) with a significance level of 0.05. A 1.2 fold change (FC) in protein abundance was further applied to statistically significant proteins to define significantly changed proteins between the control and drought-treated samples. The proteomics data are available via ProteomeXchange with identifier PXD009002.

2.7. Protein functional classification, network and subcellular location prediction

To determine the functions of the identified proteins, we searched against the annotated Uniprot database (http://www.ebi.uniprot.org) and TAIR database (http://www.arabidopsis.org/). Gene ontology analysis was performed using the singular enrichment analysis tool in AgriGo (http://bioinfo.cau.edu.cn/agriGO/) with the suggested *A. thialiana* gene model (TAIR10) as the reference. Construction of protein-protein interaction (PPI) network and extraction of significant nodes was performed using the STRING database (http://string-db.org). Subcellular localization of the identified proteins was determined as previously described [34].

2.8. Metabolite extraction from Arabidopsis leaves

Metabolite extraction was performed according to a published procedure [38]. Briefly, 100 mg FW leaf samples were homogenized with a metal ball in a screw-capped tube at 1900 strokes min^{-1} for 30 s in a GenoGrinder (Geno/Grinder 2000, SPEX SamplePrep., Metuchen, NJ, USA). Prior to extraction, 10 µl internal standard mixture of 0.1 mM lidocaine and 0.1 mM 10-camphor-sulfonic acid was added to each sample. Samples were extracted once in 1 ml of acetonitrile:isopropanol:water (3:3:2), and twice in 0.5 ml of acetonitrile: water (1:1) on a thermomixer (Thermomixer R, Eppendorf, Hamburg, Germany) at 4 °C and 1100 rpm for 5 min each time, followed by sonication for 15 min on ice and centrifugation at 13,000*g*, 4 °C for 15 min. The supernatants were combined, lyophilized to dryness, and then reconstituted in 100 µl distilled water.

2.9. Metabolites analysis using LC-multiple reaction monitoring (MRM) MS

HPLC-MRM-MS based targeted metabolite profiling was conducted using an Agilent 1100 HPLC (Agilent, Santa Clara, CA, USA) coupled with an AB Sciex 4000 QTRAP TM (AB Sciex, Framingham, MA, USA) [38]. Optimized MRM MS conditions including precursor ion, product ion, declustering potential, collision energy and cell exit potential were established for 277 authentic compounds including phytohormones [38]. For standard curves, the levels range from 0.1 pg/ml to 1 µg/ml, and the regression coefficients are > 99%. A reverse-phase C18 column (Agilent, Eclipse XDB-C18, 4.6 mm × 250 mm × 5 µm) was used for metabolite separation with 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B. The LC gradient was initially held at 1% solvent B for 5 min, then a linear gradient was imposed from 1%B to 99.5% B over 41.5 min, followed by holding at 99.5% B for 4.5 min, and then return to 1% B. The flow rate was 0.5 ml min^{-1}, and the total analysis time was 1 h. The MS conditions were: 30 psi for curtain gas, 50 psi GS1, 55 psi GS2, ion source voltage at ± 4500 V, with the turbo ESI interface temperature at 350 °C. A multiple-period (segment) method was followed as previously described [38].

2.10. Metabolic pathway mapping and enrichment analyses

Metabolic pathway analysis was performed using MetaboAnalyst 3.0 [39]. The acquired raw data were uploaded to MetaboAnalyst 3.0 webserver (www.metaboanalyst.ca), and only metabolites with KEGG identifiers were used. Differential metabolites were defined using statistical methods described previously [30]. A total of 96 metabolites were uploaded as query for the pathway enrichment and impact analysis against the *A. thaliana* metabolite database using a published protocol [39].

3. Results

3.1. Morphological, physiological and biochemical responses to drought stress

In our previous work, overepxression of *JAZ7* (OE) conferred drought tolerance in Arabidopsis [31]. To study the functions of *JAZ7* in Arabidopsis drought tolerance, we included a *JAZ7* knockout mutant (KO). As shown in Fig. 1A, the position of T-DNA insertion is in the second exon, resulting in a homozygous mutant with no detectable *JAZ7* transcript (Fig. 1B). Thus, three genotypes of plants with no expression, normal expression and overexpression of *JAZ7*, i.e., KO, WT and OE, were used in this study.

Although plate-grown young seedlings of WT and OE were indistinguishable [31], OE plants showed retarded growth in soil. At the rosette stage, OE exhibited smaller leaves with roughly a 5-day delay in development compared to those of WT and KO (Fig. 1C). At 18 days post drought (dpd) treatment, plants of three different genotypes exhibited distinct phenotypes. While the WT and KO leaves became purple (due to anthocyanin accumulation), OE leaves remained green (Fig. 1C). Due to the increased drought tolerant feature of the OE plants, they reached to a similar size of the WT and KO plants at 22 dpd, and remained green. In addition, only OE plants, but not WT and KO plant, were able to recover and grew normally after re-watering at 22 dpd (Fig. S1). Thus, OE plants showed higher drought tolerance than WT and KO plants. At 24 dpd, all plants of the three genotypes were not able to recover after re-watering. Based on these observations, seven time points (0, 6, 12, 15, 18, 21 and 24 dpd) were chosen for morphological and physiological analyses, and plants at 18 dpd were used for proteomics experiments.

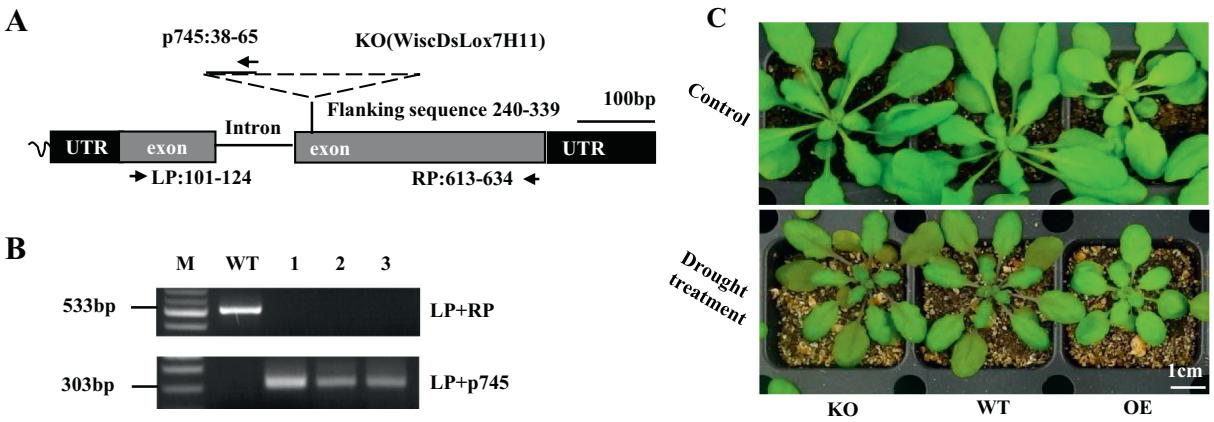

Fig. 1. Morphological phenotypes of *JAZ7* knock out (KO), wild type (WT) and overexpression (OE) plants under drought. (A) Schematic representation of the KO T-DNA insertion line (WiscDsLox7H11). The Insertion (open triangle) lies in the second exon of the *JAZ7* gene. (B) KO is a homozygous mutant of *JAZ7*, as determined by genotyping using left primer (LP) and right primer (RP) of the gene, as well as the LP and a T-DNA primer (p745). M: DNA marker; WT: wild type of *A. thaliana* Col-0; 1–3: three independent KO mutants. (C) The *JAZ7* OE plant (SALK_040835C) showed enhanced tolerance to drought comparing to KO and WT. The plants were drought-treated for 18 days.

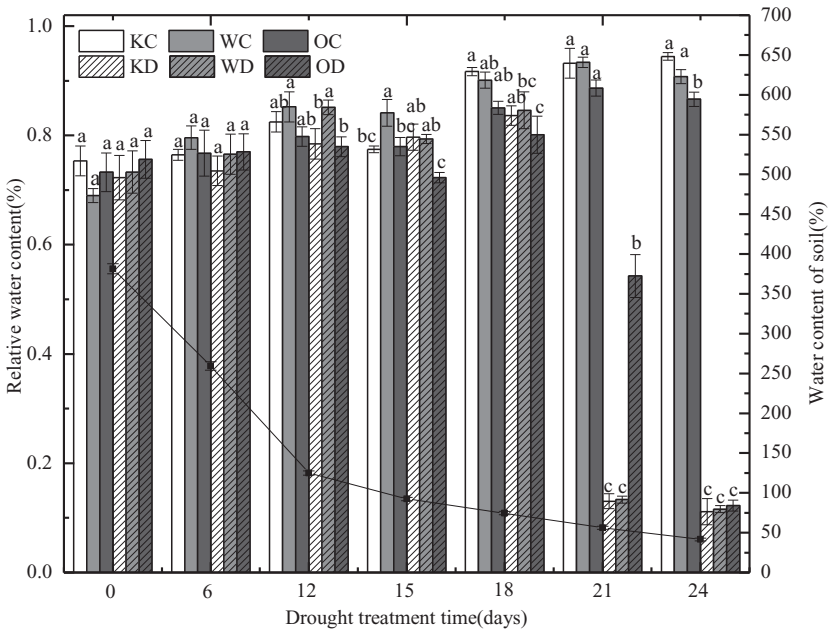

Fig. 2. Relative water content (bar graph) and soil water content (line graph) of *JAZ7* knock out (KO), wild type (WT) and overexpression (OE) plants under drought. KC, WC and OC are control groups of KO, WT and OE, respectively, with regular watering, while KD, WD and OD represent drought-treated groups. The relative water content (left y-axis) and soil water content (right y-axis) were measured at 0, 6, 12, 15, 18, 21 and 24 days post drought treatment. Values were presented as means ± SE ($n = 3$ for plants, and $n = 10$ for soil). Different letters on the bars indicate significant differences at $p \leq .05$ using the Duncan test.

Relative water content (RWC) is a good indicator of plant water status under drought stress. Leaf RWC was significantly different between OE and WT/KO plants at 18 dpd. At 21 dpd when the soil water content dropped by 75%, a striking differences in RWC was observed between the three genotypes (13%, 13% and 54% for KO, WT and OE, respectively) (Fig. 2). This result suggests that OE plants can retain more water than KO and WT. Interestingly, the OE plants had lower RWC than KO and WT from 12 days to 18 days of drought treatment. Comparing to KO and OE, WT showed significantly lower amount of chlorophyll *a* under both control and drought conditions (Fig. 3A and B). In contrast, chlorophyll *b* of WT was statistically lower than KO and OE under control, but not under drought (Fig. 3A and B). Overall, the total chlorophyll (a and b combined) showed significant increases in all the three genotypes under drought (Fig. 3C). Under drought stress, anthocyanin showed increases in all the plants (Fig. 3D). Consistent with the green leaf color of OE plants under drought (Fig. 1C), OE plants had the lowest anthocyanin content under drought stress (Fig. 3D).

3.2. Identification of differential proteins under control and drought stress

Based on the above morphological, physiological and biochemical responses to the drought treatment, Arabidopsis leaves of KO, WT and OE at 18 dpd and corresponding controls were collected for proteomics and metabolomics experiments. A shotgun proteomics workflow was utilized to compare the protein profiles in the different genotypes of plants under control and treatment (Fig. S2A). Tandem mass tag (TMT) 10-plex reagents were used to label 18 samples (3 genotypes × 2 conditions × 3 biological replicates). Small aliquots of all 18 samples were pooled to serve as a universal control to correct batch effect (see Fig. S2B for labeling scheme).

The quantitative proteomics experiments resulted in the identification of 8993 (Fig. S2C) and 2477 (Fig. S2D) proteins at 5% and 1% false discovery rate (FDR), respectively. Proteins with higher identification standard (1% FDR) were used for further data analysis. Around 60% of these proteins were identified in all the three biological replicates (Fig. S2D). Pairwise comparisons were performed to identify proteins responsive to drought in the three genotypes. Statistical analysis revealed

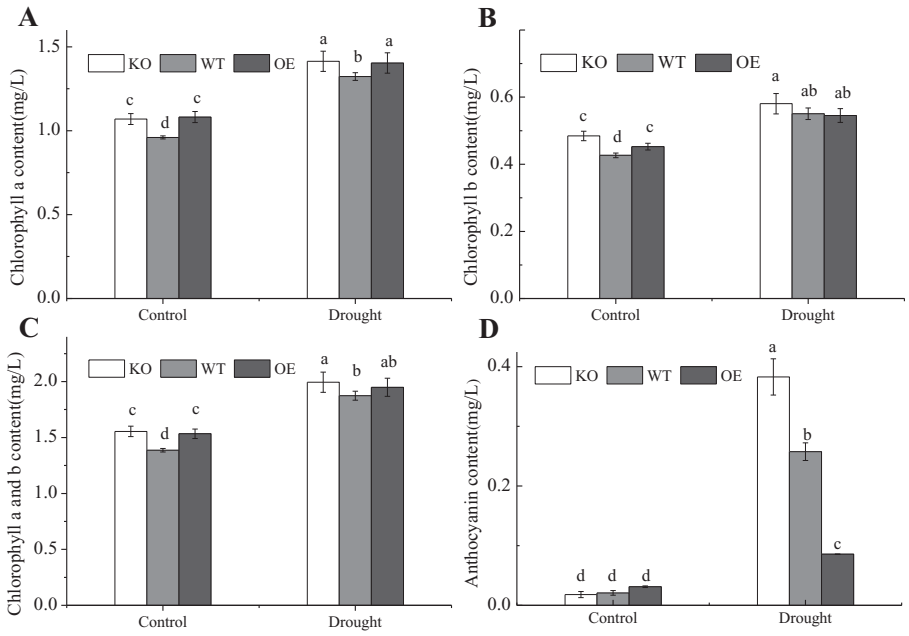

Fig. 3. Effect of drought stress on chlorophyll *a*, *b* and anthocyanin contents in *JAZ7* knock out (KO), wild type (WT) and overexpression (OE) plants. Values were presented as means ± SE (n = 3). Different letters on the bars indicate significant differences at $p \leq .05$ using the Duncan test.

that 99, 118 and 309 proteins show drought-induced changes in WT, KO and OE, respectively (Fig. 4A and B). Out of a total of 394 significantly changed proteins (Table S1), 28 were common in all the three genotypes (Fig. 4A). Consistent with the drought-resistance phenotype of the OE plants, there were substantially more differential proteins identified in the OE (with 225 unique significant proteins) comparing to the other two genotypes under drought stress. Interestingly, proteins with a decrease in abundance outnumbered proteins with an increase in abundance in all three genotypes of plants under drought (Fig. 4B). The difference in the magnitude of protein changes was shown Fig. 4C, revealing a similar change pattern between KO and WT plants and a distinct pattern of OE plants (Fig. 4C).

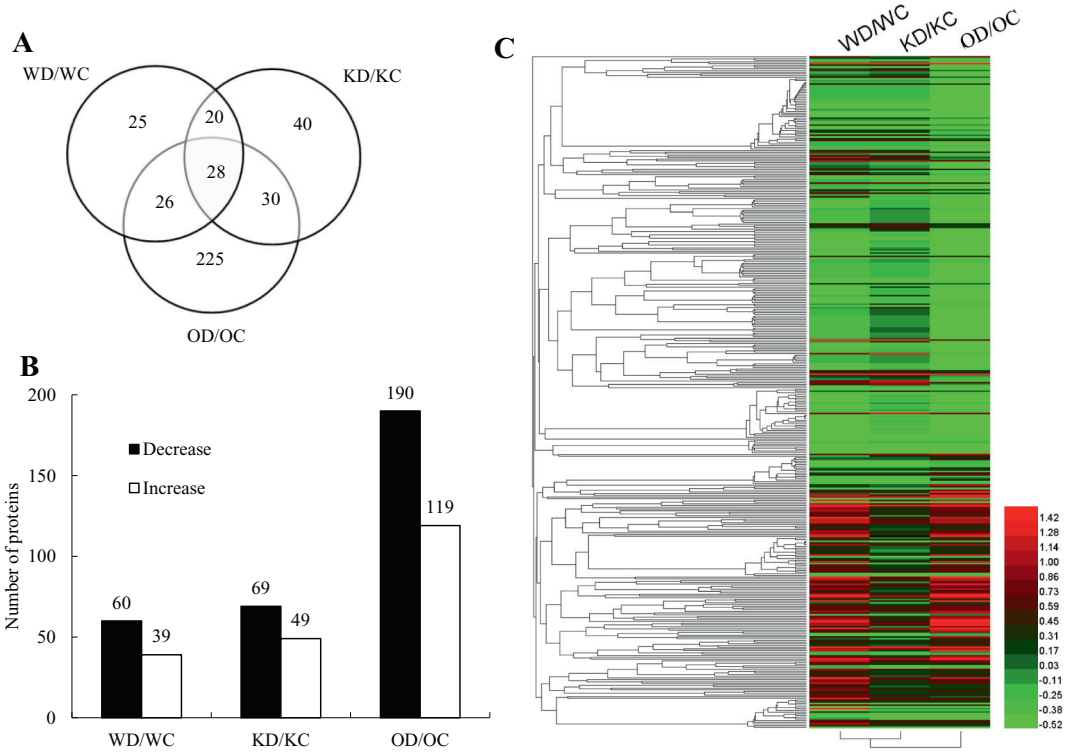

Fig. 4. Differentially expressed proteins in Arabidopsis leaves of in *JAZ7* knock out (KO), wild type (WT) and overexpression (OE) under drought stress. (A) Venn diagram showing the number of differentially expressed proteins in drought-treated plants compared to the control plants. KC, WC and OC are control groups of KO, WT and OE, respectively, with regular watering, while KD, WD and OD represent drought-treated groups. (B) Hierarchical clustering of 394 differentially expressed proteins. The color scale bar in the bottom right corner indicates increased (red) and decreased (green) levels, and no significant changes (black) of proteins in response to drought stress. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

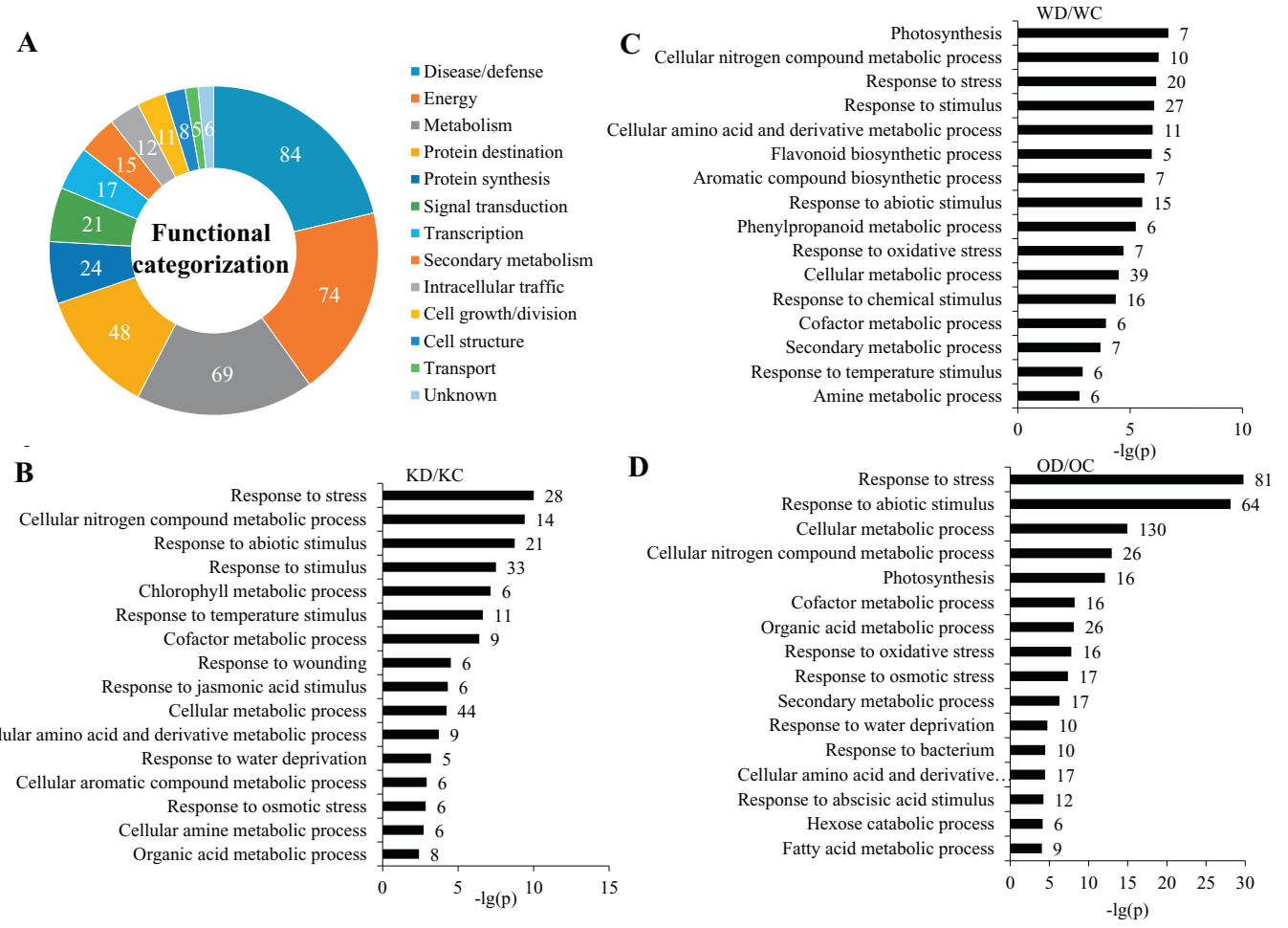

Fig. 5. Functional classification and pathway enrichment of the differentially expressed proteins in *JAZ7* knock out (KO), wild type (WT) and overexpression (OE) plants under drought. (A) A total of 394 differential proteins were classified into eight functional categories. (B)-(C) Enriched pathways under drought stress in KO, WT and KO plants, respectively. KC, WC and OC are control groups of KO, WT and OE, respectively, with regular watering, while KD, WD and OD represent drought-treated groups. Singular enrichment analysis in AgriGo with the *A. thialiana* gene model (TAIR10) was used as the reference.

3.3. Functional analysis of differential proteins under drought

To determine which functional categories were strongly related to the drought stress response, the 394 differentially expressed proteins were classified according to their predicted or known functions. The resulting 13 categories included disease/defense (84), energy (74), metabolism (69), protein destination (48), protein synthesis (24), signal transduction (21), transcription (17), secondary metabolism (15), intracelluar traffic (12), cell growth/division (11), cell structure (8), transport (5) and unknown (6) (Fig. 5A). About half of the proteins belonged to the functional categories of disease/defense, energy and metabolism. In terms of subcellular localization, 210 significantly changed proteins were predicted to localize in chloroplast, 82 in cytoplasm, 28 in mitochondria, 18 in nucleus, 9 in apoplast, 9 in vacuole, 7 in membrane,7 secreted, 6 in endoplasmic reticulum, 5 in golgi, 4 in peroxisome, and 2 in cell wall (Fig. S2E). This result suggests that our proteomics workflow is not biased toward proteins in certain cellular compartments and that proteins in various subcellular locations responded to drought stress.

Pathway enrichment was performed to reveal significant biological processes in response to drought in the KO, WT and OE plants. As shown in Fig. 5B–D, GO terms such as responses to stimulus, cellular process, and metabolic process were enriched in all the three genotypes under drought stress. As expected, the OE plants had the largest number of genes in each GO term, while KO and WT had similar numbers. For example, the numbers of proteins in the category of "response to stress" were 81, 28 and 20 for OE, KO and WT, respectively (Fig. 5B–D). Thus, overexpression of *JAZ7* caused a profound impact on the proteome, while knocking out of *JAZ7* had little effect. These results were in line with our observation of the enhanced drought tolerance in OE plants and the similar drought responses in KO and WT plants. Interestingly, the effect of *JAZ7* knockout on the proteome level was also detected. For example, six proteins related to "response to jasmonic acid (JA) stimulus" were found in the KO, but not in WT plants (Fig. 5B, C). In JA biosynthesis, the three genotypes also showed different protein expression patterns under the drought treatment (Fig. 6). Overexpression of *JAZ7* resulted in an increase of (acyl-carrier-protein)-S-malonyltransferase, Acetoacetyl- acyl-carrier-protein, 3-oxoacyl-acyl-carrier-protein reductase, allen oxide synthase (AOS), lipoxygenase (LOX) of the JA synthesis pathway under drought. In contrast, only LOX and AOS showed increases under drought in WT and KO, respectively. This result may indicate a direct feedback regulation of JA synthesis due to the suppression of JA signaling pathway by *JAZ7* overexpression.

To reveal the protein network of these significantly changed proteins, we constructed protein-protein interaction (PPI) networks in the three different genotypes. Clearly, the OE plants showed the most nodes and edges, followed by KO and WT (Fig. S3). These data indicate that *JAZ7* overexpression resulted in dramatic changes in the proteome and interactome, and thus drought stress tolerance of the plants.

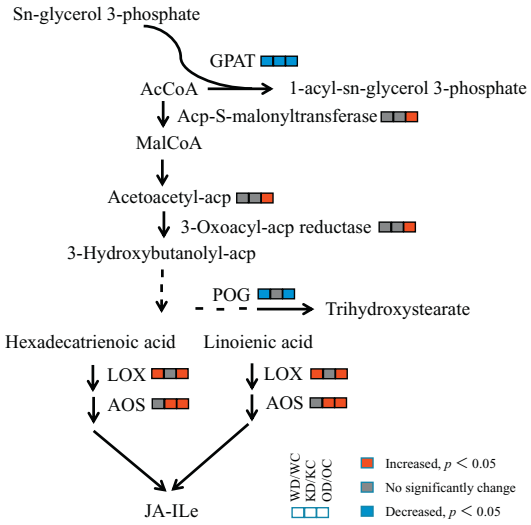

Fig. 6. Changes of proteins in JA biosynthesis in wild type (WT), *JAZ7* knock out (KO) and overexpression (OE) plants revealed by proteomics. Red and blue denote increased and decreased proteins, respectively, in response to drought. KC, WC and OC are control groups of KO, WT and OE, respectively, with regular watering, while KD, WD and OD represent drought-treated groups. Abbreviations: AOS, allen oxide synthase; LOX, lipoxygenase; POG, peroxygenase; GPAT, glycerol-3-phosphate acyltransferase; (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Metabolite profiling reveals both common and unique changes under drought

In order to profile metabolite changes in the OE, WT and KO plants under drought stress, we extracted metabolites from Arabidopsis leaves at 18 dpd and analyzed the samples on our HPLC-MRM-MS system [40]. A total of 238 metabolites were identified and quantified, 214 have peak area values in all the samples (Table S2). They covered both primary and specialized metabolic pathways, including in fatty acid, flavonoid, phenylpropanoid, amino acid, citric acid, purine and pyrimidine metabolism. As shown in Fig. 7A, OE, WT and KO had 42, 54 and 45 differential metabolites, respectively. While there were 11 significant metabolites identified from all three genotypes, 60 significant metabolites were unique to WT, KO or OE plants. Thus, both common and unique changes in metabolic pathways were involved in drought response in Arabidopsis plants with different expression levels of *JAZ7*. Among all the 96 significantly changed metabolites (Table S2), WT and OE plants had similar numbers of decreased and increased metabolites. In contrast, majority of the significantly changed metabolites in KO decreased and only four increased in KO after drought stress (Fig. 7B). Amino acids and antioxidant metabolites account for most of the significantly changed metabolites (Fig. S4). Hierarchical cluster analysis showed that the patterns of metabolite changes are dependent on genotypes (Fig. S4). For example, amino acid such as threonine showed decreases in WT and OE lines, but an increase in KO plants under drought. The highly branched clustering patterns may indicate a complexed interaction between genotype and drought for metabolite accumulation.

All the metabolites affected by drought were subjected to pathway analysis by MetaboAnalyst. Fig. 7C showed most relevant biological

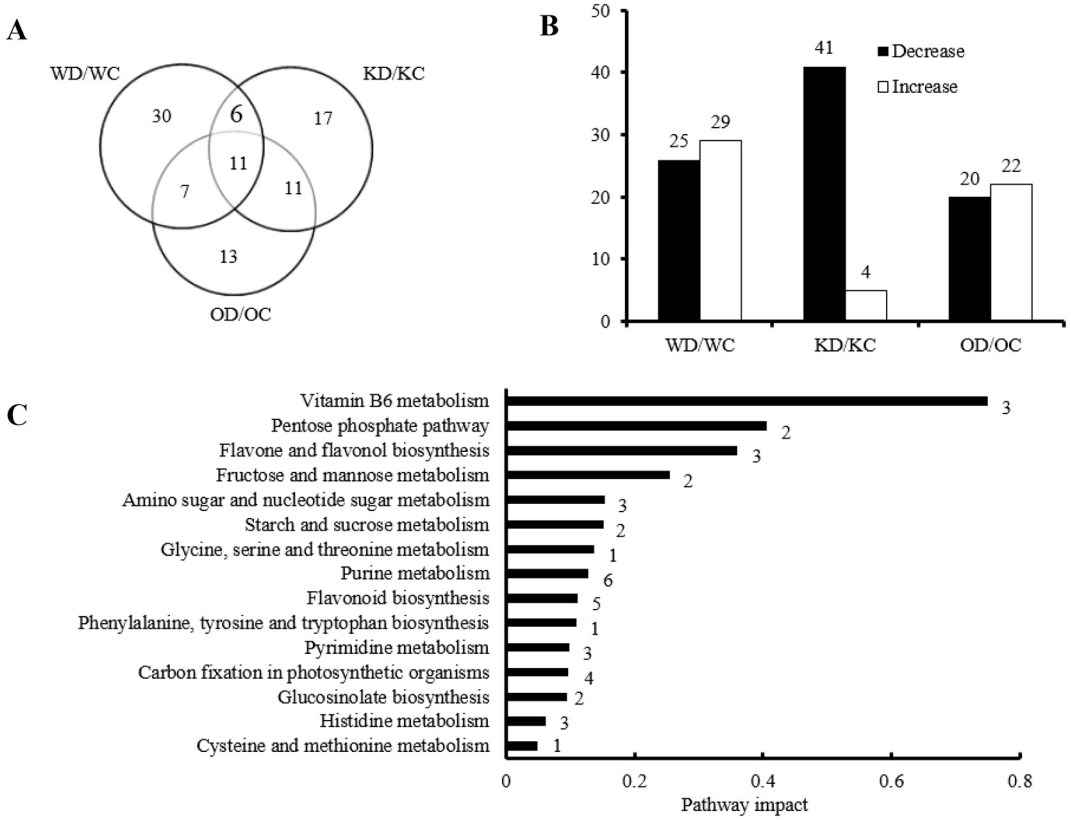

Fig. 7. Differential metabolites in Arabidopsis leaves of wild type (WT), *JAZ7* knock out (KO) and overexpression (OE) plants under drought. (A) A venn diagram showing the number of metabolites with differential levels in stressed plants compared to control plants. KC, WC and OC are control groups of KO, WT and OE, respectively, with regular watering, while KD, WD and OD represent drought-treated groups. (B) Number of decreased or increased metabolites in each genotype under control or drought treatment. (C) Pathway enrichment of differentially accumulated metabolites under drought. The pathway impact on the x axis indicates the extent to which the pathway is influenced, and the numbers next to each bar of a specific pathway denote the numbers of metabolites profiled.

pathways in response to drought stress. While the pathway impact on the x axis indicates the extent to which the pathway is influenced, the numbers next to each bar of a specific pathway denote the numbers of metabolites profiled in this study. The enrichment of primary pathways such as vitamin B6 metabolism, pentose phosphate pathway, fructose and mannose metabolism, and amino sugar and nucleotide sugar metabolism indicated that these pathways are responsive to drought stress. In addition, secondary metabolite pathways, such as flavone, flavonol and flavonoid biosynthesis, were enriched under drought treatment. Since only a few proteins involved in the metabolism of pentose phosphate, fructose, and flavonoid were identified (Table S1), targeted proteomics and activity assays are needed for validating the metabolomics findings. This result also highlights the complementary nature of current proteomics and metabolomics approaches.

4. Discussion

Water deficit is a major limiting factor for plant growth and yield in arid areas. Enhancing plant drought tolerance will help plants overcome the limitation and thus contribute to crop production and global food security. With the goal to decipher the molecular mechanisms underlying the drought tolerance conferred by *JAZ7* overexpression, we conducted an integrated proteomics and metabolomics study. The results showed that overexpression of *JAZ7* led to drastic changes in both the proteome and metabolome in the OE plants under drought stress. Biological processes such as photosynthesis, redox regulation and metabolism are involved in JAZ7 mediated drought tolerance in OE plants.

4.1. JAZ7 OE enhances drought tolerance through HSP and photosynthesis regulation

Our physiological data clearly showed that overexpression of *JAZ7* enhanced drought tolerance (Fig. 1). Proteomics revealed that multiple mechanisms including regulation of ATP synthesis, photosynthesis/carbon fixation, glycolysis and stress and defense are involved. For example, carotenoid cleavage dioxygenase, dehydrin family protein, heat shock proteins (HSPs), desiccation-responsive protein 29A (RD29A), glyceraldehyde-3-phosphate dehydrogenase, and lactate/malate dehydrogenase family protein increased dramatically in the OE plants. In WT, bisphosphate nucleotidase, soluble epoxide hydrolase and granulin repeat cysteine protease family protein increased. In KO, only granulin repeat cysteine protease family protein increased. Some of these proteins may be directly related to the OE enhanced drought tolerance. For example, among the 394 differential proteins, a mitochondrial 23.6 kDa HSP showed a 19 fold increase in both OE and WT. Importantly, OE had five HSPs changed dramatically, while WT had one and KO had no significant changed HSPs (Table S1). Production of high levels of heat shock proteins may help maintain protein stability and reduce drought damage to cellular structure and biochemical machinery.

Photosynthesis plays a central role in plant energy metabolism. Its efficiency was shown to be dramatically reduced by drought. In this study, 44 proteins involved in photosynthesis showed differential expression levels in the three genotypes, and OE alone had 39 differential photosynthesis proteins (Table S1). For example, Rubisco activase (involved in the first major step of carbon fixation) decreased remarkably in response to drought, similar to other proteomics results [41–43]. To cope with the drought condition, OE also decreased proteins in photosystem II (PS II) including the reaction center protein H, reaction center PSB28, protein subunit Q-2, reaction center protein E, and oxygen-evolving enhancer protein 3. Down-regulation of the PSII system may help with water conservation [44,45]. In contrast, WT had one increased and four decreased proteins in the Photosystem II. KO plants did not show any differential expressed proteins in PS II, indicating JAZ7 plays an important role in drought-induced inhibition of PS II. It is worth noting that not all the photosynthesis proteins decreased. PsaD, which forms complexes with ferredoxin and ferredoxin-oxidoreductase in PS I, increased in all the three genotypes. This suggests that more PsaD is recruited to assist the electron transfer from PS I to ferredoxin to restore redox homeostasis under drought [46]. Other PS I proteins showed genotype-dependent changes under drought. For example, subunit G decreased only in WT and OE, and subunit PsaN decreased only in OE. The function of PsaN in drought stress remains unknown.

4.2. JAZ7 is involved in redox regulation in plant drought response

Redox regulation plays an important role in plant stress biology [47,48]. To reduce ROS caused damage under drought stress, several ROS scavenging enzymes, such as superoxide dismutase (SOD) and glutathoine S-transferase (GST) exhibited different expression levels in the three genotypes. WT and KO showed decreased Fe-Mn family of SOD, while OE had decreased Cu-Zn SOD and KO showed increased ascorbate peroxidase (APX). The enzyme related to glutathione metabolism also changed dramatically. For example, lactoylglutathione lyase (LGL) increased in WT and KO , but it decreased in OE together with a hydroxyacylglutathione hydrolase (HGH). LGL converts methylglyoxal (MG) and glutathione to form S-lactoylglutathione. HGH follows LGL, and both act together to remove the potentially toxic metabolite MG and related compounds [49,50]. Interestingly, both glutathione dehydrogenase and glutathione reductase were significantly increased in the OE plants (Table S1), indicating high reducing environment in the OE cells. This may allow OE plants to maintain MG at very low levels, contributing to drought tolerance. Alternatively, JAZ7 may be regulated as a result of perturbation in redox status. Although the activities of some proteins have been shown to be under redox control [51–53], whether JAZ7 undergoes redox-induced change in transcription repressor activity remains unknown.

Our metabolomics study also identified differential accumulation patterns of metabolites that are involved in redox regulation, as antioxidant accumulation is important for plants to cope with drought [35]. Some antioxidants showed differential changes among the genotypes. For example, pyridoxal 5-phosphate is an essential quencher of singlet oxygen in plants, and it protects cellular membranes from lipid peroxidation. Pyridoxal 5-phosphate, pyridoxine and pyridoxine dihydrochloride decreased in WT and OE, but increased in KO. The decrease of pyridoxal 5-phosphate is consistent with the protein level change of pyridoxal 5′-phosphate synthase pdxS subunit. In addition, sarcosine important in betaine biosynthesis increased in KO and WT, but decreased in OE (Table S2). These results suggest that different strategies were deployed in OE to cope with drought stress.

4.3. Drought-induced changes in JA pathways, amino acids, phytohormones and glucosinolates

JA pathway proteins were drought-responsive in our proteomics data. They include key enzymes in JA biosynthesis pathways such as allene oxide synhase (AOS) and lipoxygenase 2 (LOX2), as well as proteins in JA signaling such as L-cystine betalyase (CORI3) [20]. CORI3 increased expression in the three genotypes, indicating a common response protein under the drought condition. CORI3 was found to interact with COI, an important component of the JA signaling pathway [54]. CORI3 was also influenced by sulfate availability and ABA in response to drought [3,4]. In the protein interaction networks, CORI3 was a common node in the three different genotypes, but it had different edges (Fig. 3). The different interaction networks may account for the drought tolerance differences among the OE, WT and KO plants. Our metabolomics data also identified several JA and its derivatives (Fig. S4). However, no significant difference in their abundance was found among genotypes under the control and drought stress. The inconsistency between the protein-level regulation of JA (Fig. 6) and metabolite-level of JA (Fig. S4) may indicate that JA level changes may

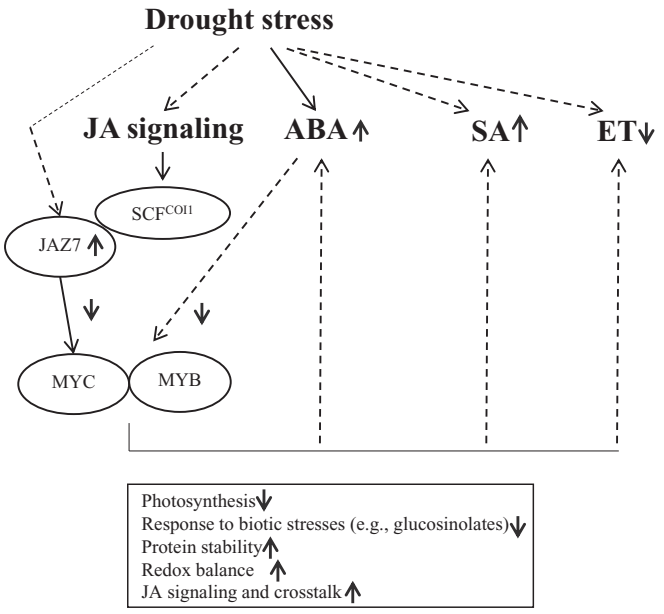

Fig. 8. A working model depicting JAZ7-mediated drought tolerance. *JAZ7* overexpression led to phytohormone changes, enhancing protein stability and redox maintenance, and decreasing of photosynthesis and response to biotic stresses, and ultimately plant drought tolerance phenotype.

not be a strategy of OE plants to cope with drought stress. However, JA signal transduction involving protein changes may be important. For example, secondary metabolites that are responsive to JA were differentially accumulated in these plants (Fig. 7).

Using targeted LC-MRM-MS, a total of 96 metabolites were found significantly changed under drought (Table S2). The increases of many metabolites have been demonstrated to provide osmoprotection, maintain enzyme activity, and quench ROS. Increase of amino acid concentrations was closely related to the rapid adjustment of water availability in *Calotropis procera*, *Hordeum vulgare* and eggplant under drought stress [2,55,56] or salt stress [9,55]. In this study, the levels of 3-methyl-L-histidine, asparagine, histidine, homomethionine, threonine and 3-methyl-L-histidine increased in all the three genotypes, but KO accumulated more amino acids than the other two genotypes. For examples, leucine, threonine, asparagine and homomethionine showed increases only in the KO. The increase of amino acids may stem from amino acid synthesis or stress-induced protein breakdown. Accumulation of amino acids was suggested to facilitate plant stress tolerance through osmotic adjustment and ROS detoxification [55]. However, in KO and WT, the accumulation of amino acids might indicate cellular damage and protein degradation under drought [57].

A precursor of ethylene (1-aminocyclopropane-1-carboxylic acid) increased in WT under drought stress, but not in OE (Table S2). This is consistent with the decrease of the key enzyme S-adenosylmethionine synthetase 1 in ethylene synthesis. In addition to ethylene, methoxy cinnamic acid, the precursor of salicylic acid (SA) increased in both WT and OE. Since *JAZ7* overexpression may repress the JA signaling, it may be expected that SA synthesis increased due to the well-known antagonism between the SA and JA [4]. Although the JA-related proteins increased in OE, JAs did not show significant variations between drought and control in the three genotypes (Fig. S5). Interestingly, a cytokinin kinetin increased dramatically only in OE, and it can prevent protein breakdown and activate protein synthesis. This data correlate well with the enhanced protein synthesis in OE plants under drought stress (Table S1). In addition, guanosine increased dramatically in OE. It may be involved as a precursor of guanosine nucleotides in the ABA induced stomatal closure process [58]. The amount of ABA glucose ester decreased dramatically in WT and OE, but not in KO. ABA is an important phytohormone that plays a critical role in response to drought stress [59]. Drought can trigger activation of ABA synthesis and inhibition of its degradation (as indicated by the decreases of phaseic acid), resulting in the ABA accumulation [35,54,58]. One of the ABA biosynthesis enzyme 9-cis-epoxycarotenoid dioxygenase showed a two fold increase in OE. However, the active form of ABA did not show significant differences under the drought stress. Interestingly, OE showed a little slower germination than the other two genotypes. Under short day, OE also showed delayed flowering time (Fig. S3). It appeared that *JAZ7* overexpression may result in changes of ABA sensitivity.

In all the three genotypes, syringic acid, sinapinic acid and malonic acid increased, and anthranilic acid decreased under drought (Table S2). Syringic acid and sinapinic acid are phenolic antioxidants that can efficiently scavenge radicals [60]. Malonic acid is the product induced by γ-aminobutyric acid, which has function in stress resistance [61]. Anthranilic acid is the precursor of tryptophan, which can be metabolized to produce glucosinolates and auxin. In addition, homomethionine (a precursor of aliphatic glucosinolates) decreased in WT and OE plants, but increased in KO under drought stress (Table S2). The decreases of anthranilic acid and homomethionine indicate that lowering defense related specialized metabolites may enhance drought tolerance [8,41]. Furthermore, the two glucosinolate biosynthesis enzymes, desulfo-glucosinolate sulfotransferase and 2-oxoglutarate (2OG)/iron-dependent dioxygenase, showed lower levels under drought than under control conditions (Table S1). The sulfotransferase utilizes 3′-phospho-5′-adenylyl sulfate (PAPS) as donor to catalyze the sulfate conjugation of desulfo-glucosinolates (dsGSs), and constitutes the final step in the biosynthesis of the glucosinolate core structure. Glucosinolates are known to be important for pathogen and insect defense [62]. By lowering the levels of these proteins, OE plants may be able to allocate more resources for dealing with the abiotic stress. Clearly, overexpression of *JAZ7* modulates various biological processes including photosynthesis, redox and response to biotic stress, leading to significant changes of antioxidants and amino acids, phytohormones and defense metabolites (Fig. 8).

5. Conclusions

In this study, we investigated the contribution of JAZ7 in drought responses through proteomics and metabolomics of *JAZ7* KO, WT and OE plants. Key drought-responsive proteins and metabolites were identified and quantified. OE plants showed more changes in both the proteome and the metabolic profile than the other two genotypes. It has become clear that *JAZ7* overexpression modulates photosynthesis, redox and response to biotic stress. In addition, overexpression of this transcription repressor also brought about major reprogramming of metabolic pathways, including those of antioxidants and amino acids, phytohormones and defense metabolites. Collectively, these observed molecular level changes may explain the observed drought-tolerance phenotype of the *JAZ7* OE plants. Our data also complemented the notion that JAZ7 protein acts as a key regulator in the cross-talk between JA and other hormones (e.g., SA and ABA).

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2019.02.001.

Acknowledgments

This work was partially supported by a U.S. National Science Foundation (NSF) grant MCB 0845162 to SC, and a NSF REU grant 1560049 for the support of Peter Scott to carry out part of this research. The authors thank Ning Zhu in the Proteomics and Mass Spectrometry Core, Interdisciplinary Center for Biotechnology Research, University of Florida for assistance, Zepeng Yin at Shenyang Agricultural University, Bing Yu and Huizi Duanmu at Heilongjiang University for helpful discussions.

Author contribution

L.M performed the experiments and wrote the manuscript draft; T.Z performed proteomics experiments, data analysis and manuscripts revision. S.G performed metabolites experiments and data analysis. P.S. helped with data analysis and edited the manuscript. H.L. provided guidance. S.C. designed the experiments, provided guidance and finalized the manuscript.

Conflict of interest

All authors of this manuscript declare that they have no conflict of interest.

Declarations

The corresponding authors certify that all authors have seen and approved the final version of the manuscript being submitted and warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

References

[1] D. Todaka, K. Shinozaki, K. Yamaguchi-Shinozaki, Recent advances in the dissection of drought-stress regulatory networks and strategies for development of drought-tolerant transgenic rice plants, Front. Plant Sci. 6 (2015) 84.

[2] A. AghaKouchak, L. Cheng, O. Mazdiyasni, A. Farahmand, Global warming and changes in risk of concurrent climate extremes: Insights from the 2014 California drought, Geophys. Res. Lett. 41 (2014) 8847–8852.

[3] S.K. Sah, K.R. Reddy, J. Li, Abscisic acid and abiotic stress tolerance in crop plants, Front. Plant Sci. 7 (2016) 571.

[4] V. Verma, P. Ravindran, P.P. Kumar, Plant hormone-mediated regulation of stress responses, BMC Plant Biol. 16 (2016) 86.

[5] X. Nxele, A. Klein, B.K. Ndimba, Drought and salinity stress alters ROS accumulation, water retention, and osmolyte content in sorghum plants, S. Afr. J. Bot. 108 (2017) 261–266.

[6] Z. Wang, F. Wang, Y. Hong, J. Huang, H. Shi, J.K. Zhu, Two chloroplast proteins suppress drought resistance by affecting ROS production in guard cells, Plant Physiol. 172 (2016) 2491–2503.

[7] I. Aranjuelo, G. Molero, G. Erice, J.C. Avice, S. Nogues, Plant physiology and proteomics reveals the leaf response to drought in alfalfa (*Medicago sativa* L.), J. Exp. Bot. 62 (2011) 111–123.

[8] D.W. Lawlor, W. Tezara, Causes of decreased photosynthetic rate and metabolic capacity in water-deficient leaf cells: a critical evaluation of mechanisms and integration of processes, Ann. Bot. 103 (2009) 561–579.

[9] S. Ramanjulu, C. Sudhakar, Drought tolerance is partly related to amino acid accumulation and ammonia assimilation: a comparative study in two mulberry genotypes differing in drought sensitivity, J. Plant Physiol. 150 (1997) 345–350.

[10] C. Liang, Ascorbate peroxidase gene from Brassica napus enhances salt and drought tolerances in *Arabidopsis thaliana*, Afr. J. Biotechnol. 10 (2011) 18085–18091.

[11] J. Csiszar, A. Galle, E. Horvath, P. Dancso, M. Gombos, Z. Vary, L. Erdei, J. Gyorgyey, I. Tari, Different peroxidase activities and expression of abiotic stress-related peroxidases in apical root segments of wheat genotypes with different drought stress tolerance under osmotic stress, Plant Physiol. Biochem. 52 (2012) 119–129.

[12] J.K. Zhu, Salt and drought stress signal transduction in plants, Annu. Rev. Plant Biol. 53 (2002) 247–273.

[13] A. Roldan, P. Diaz-Vivancos, J.A. Hernandez, L. Carrasco, F. Caravaca, Superoxide dismutase and total peroxidase activities in relation to drought recovery performance of mycorrhizal shrub seedlings grown in an amended semiarid soil, J. Plant Physiol. 165 (2008) 715–722.

[14] J.H. Chen, H.W. Jiang, E.J. Hsieh, H.Y. Chen, C.T. Chien, H.L. Hsieh, T.P. Lin, Drought and salt stress tolerance of an Arabidopsis glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid, Plant Physiol. 158 (2012) 340–351.

[15] S. Neill, R. Barros, J. Bright, R. Desikan, J. Hancock, J. Harrison, P. Morris, D. Ribeiro, I. Wilson, Nitric oxide, stomatal closure, and abiotic stress, J. Exp. Bot. 59 (2008) 165–176.

[16] J. Yao, C. Mecey, M. Melotto, W. Zeng, S. He, The bacterial phytotoxin coronatine targets the Arabidopsis SCF(COI1)-JAZ protein complex, Phytopathology 98 (2008) S177.

[17] K. Kazan, Diverse roles of jasmonates and ethylene in abiotic stress tolerance, Trends Plant Sci. 20 (2015) 219–229.

[18] J. Browse, G.A. Howe, New weapons and a rapid response against insect attack, Plant Physiol. 146 (2008) 832–838.

[19] M. de Torres Zabala, B. Zhai, S. Jayaraman, G. Eleftheriadou, R. Winsbury, R. Yang, W. Truman, S. Tang, N. Smirnoff, M. Grant, Novel JAZ co-operativity and unexpected JA dynamics underpin Arabidopsis defence responses to Pseudomonas syringae infection, New Phytol. 209 (2016) 1120–1134.

[20] C. Wasternack, B. Hause, Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany, Ann. Bot. 111 (2013) 1021–1058.

[21] B. Thines, L. Katsir, M. Melotto, Y. Niu, A. Mandaokar, G. Liu, K. Nomura, S.Y. He, G.A. Howe, J. Browse, JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling, Nature 448 (2007) 661–665.

[22] L. Pauwels, A. Goossens, The JAZ proteins: a crucial interface in the jasmonate signaling cascade, Plant Cell 23 (2011) 3089–3100.

[23] O. Lorenzo, J.M. Chico, J.J. Sanchez-Serrano, R. Solano, JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis, Plant Cell 16 (2004) 1938–1950.

[24] T. Doerks, R.R. Copley, J. Schultz, C.P. Ponting, P. Bork, Systematic identification of novel protein domain families associated with nuclear functions, Genome Res. 12 (2002) 47–56.

[25] A. Chini, S. Fonseca, J.M. Chico, P. Fernandez-Calvo, R. Solano, The ZIM domain mediates homo- and heteromeric interactions between Arabidopsis JAZ proteins, Plant J. 59 (2009) 77–87.

[26] S. Kagale, M.G. Links, K. Rozwadowski, Genome-wide analysis of ethylene-responsive element binding factor-associated amphiphilic repression motif-containing transcriptional regulators in Arabidopsis, Plant Physiol. 152 (2010) 1109–1134.

[27] L. Pauwels, G.F. Barbero, J. Geerinck, S. Tilleman, W. Grunewald, A.C. Perez, J.M. Chico, R.V. Bossche, J. Sewell, E. Gil, G. Garcia-Casado, E. Witters, D. Inze, J.A. Long, G. De Jaeger, R. Solano, A. Goossens, NINJA connects the co-repressor TOPLESS to jasmonate signalling, Nature 464 (2010) 788–791.

[28] L.F. Thatcher, V. Cevik, M. Grant, B. Zhai, J.D. Jones, J.M. Manners, K. Kazan, Characterization of a *JAZ7* activation-tagged Arabidopsis mutant with increased susceptibility to the fungal pathogen *Fusarium oxysporum*, J. Exp. Bot. 67 (2016) 2367–2386.

[29] J. Yu, Y. Zhang, C. Di, Q. Zhang, K. Zhang, C. Wang, Q. You, H. Yan, S.Y. Dai, J.S. Yuan, W. Xu, Z. Su, JAZ7 negatively regulates dark-induced leaf senescence in Arabidopsis, J. Exp. Bot. 67 (2016) 751–762.

[30] T. Zhang, L. Meng, W. Kong, Z. Yin, Y. Wang, J.D. Schneider, S. Chen, Quantitative proteomics reveals a role of JAZ7 in plant defense response to *Pseudomonas syringae* DC3000, J. Proteome 175 (2018) 114–126.

[31] H. Yan, M.J. Yoo, J. Koh, L. Liu, Y. Chen, D. Acikgoz, Q. Wang, S. Chen, Molecular reprogramming of Arabidopsis in response to perturbation of jasmonate signaling, J. Proteome Res. 13 (2014) 5751–5766.

[32] Z. Yin, J. Ren, L. Zhou, L. Sun, J. Wang, Y. Liu, X. Song, Water deficit mechanisms in perennial shrubs *Cerasus humilis* leaves revealed by physiological and proteomic analyses, Proteome Sci. 15 (2016) 9.

[33] C. Parry, J.M. Blonquist Jr., B. Bugbee, In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship, Plant Cell Environ. 37 (2014) 2508–2520.

[34] S. Wei, Y. Bian, Q. Zhao, S. Chen, J. Mao, C. Song, K. Cheng, Z. Xiao, C. Zhang, W. Ma, H. Zou, M. Ye, S. Dai, Salinity-induced palmella formation mechanism in halotolerant algae *Dunaliella salina* revealed by quantitative proteomics and phosphoproteomics, Front. Plant Sci. 8 (2017) 810.

[35] R. Nakabayashi, K. Yonekura-Sakakibara, K. Urano, M. Suzuki, Y. Yamada, T. Nishizawa, F. Matsuda, M. Kojima, H. Sakakibara, K. Shinozaki, A.J. Michael, T. Tohge, M. Yamazaki, K. Saito, Enhancement of oxidative and drought tolerance in Arabidopsis by overaccumulation of antioxidant flavonoids, Plant J. 77 (2014) 367–379.

[36] T. Zhang, J.D. Schneider, N. Zhu, S. Chen, Identification of MAPK substrates using quantitative phosphoproteomics, Methods Mol. Biol. 1578 (2017) 133–142.

[37] T. Zhang, M. Zhu, N. Zhu, J.M. Strul, C.P. Dufresne, J.D. Schneider, A.C. Harmon, S. Chen, Identification of thioredoxin targets in guard cell enriched epidermal peels using cysTMT proteomics, J. Proteome 133 (2016) 48–53.

[38] S. Geng, B.B. Misra, E. de Armas, D.V. Huhman, H.T. Alborn, L.W. Sumner, S. Chen, Jasmonate-mediated stomatal closure under elevated CO_2 revealed by time-resolved metabolomics, Plant J. 88 (2016) 947–962.

[39] J. Xia, I.V. Sinelnikov, B. Han, D.S. Wishart, MetaboAnalyst 3.0-making metabolomics more meaningful, Nucleic Acids Res. 43 (2015) W251–W257.

[40] X. Jin, R.S. Wang, M. Zhu, B.W. Jeon, R. Albert, S. Chen, S.M. Assmann, Abscisic acid-responsive guard cell metabolomes of Arabidopsis wild-type and *gpa1* G-protein mutants, Plant Cell 25 (2013) 4789–4811.

[41] A.E. Carmo-Silva, A.J. Keys, P.J. Andralojc, S.J. Powers, M.C. Arrabaca, M.A. Parry, Rubisco activities, properties, and regulation in three different C4 grasses under drought, J. Exp. Bot. 61 (2010) 2355–2366.

[42] J.A. Rollins, E. Habte, S.E. Templer, T. Colby, J. Schmidt, M. von Korff, Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.), J. Exp. Bot. 64 (2013) 3201–3212.

[43] J.A. Perdomo, S. Capo-Bauca, E. Carmo-Silva, J. Galmes, Rubisco and Rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit, Front. Plant Sci. 8 (2017) 15.

[44] M.M. Minnaar, R.J. Strasser, G.H.J. Kruger, Constraints on PS II function and CO_2 assimilation of the C4 crop, *Zea mays*, imposed by SO_2 and drought, S. Afr. J. Bot. 77 (2011) 573–574.

[45] A.J. Pieters, S. El Souki, Effects of drought during grain filling on PS II activity in rice, J. Plant Physiol. 162 (2005) 903–911.

[46] M. Hansson, A.V. Vener, Identification of three previously unknown in vivo protein phosphorylation sites in thylakoid membranes of *Arabidopsis thaliana*, Mol. Cell.

Proteomics 2 (2003) 550–559.
[47] K.M. Balmant, T. Zhang, S. Chen, Protein phosphorylation and redox modification in stomatal guard cells, Front. Physiol. 7 (2016) 26.
[48] G. Noctor, A. Mhamdi, C.H. Foyer, The roles of reactive oxygen metabolism in drought: not so cut and dried, Plant Physiol. 164 (2014) 1636–1648.
[49] Z.G. Li, X.Q. Duan, X. Min, Z.H. Zhou, Methylglyoxal as a novel signal molecule induces the salt tolerance of wheat by regulating the glyoxalase system, the antioxidant system, and osmolytes, Protoplasma 254 (2017) 1995–2006.
[50] T.S. Hoque, M.A. Hossain, M.G. Mostofa, D.J. Burritt, M. Fujita, L.S. Tran, Methylglyoxal: an emerging signaling molecule in plant abiotic stress responses and tolerance, Front. Plant Sci. 7 (2016) 1341.
[51] T. Ma, M.J. Yoo, T. Zhang, L. Liu, J. Koh, W.Y. Song, A.C. Harmon, W. Sha, S. Chen, Characterization of thiol-based redox modifications of *Brassica napus* SNF1-related protein kinase 2.6-2C, FEBS Open Biol. 8 (2018) 628–645.
[52] M. Zhu, T. Zhang, W. Ji, C. Silva-Sanchez, W.Y. Song, S.M. Assmann, A.C. Harmon, S. Chen, T. Zhang, M. Zhu, W.Y. Song, A.C. Harmon, S. Chen, Redox regulation of a guard cell SNF1-related protein kinase in *Brassica napus*, an oilseed crop, Biochem. J. 474 (2017) 2585–2599.
[53] T. Zhang, M. Zhu, W.Y. Song, A.C. Harmon, S. Chen, Oxidation and phosphorylation of MAP kinase 4 cause protein aggregation, Biochim. Biophys. Acta 1854 (2015) 156–165.
[54] L.B. Sheard, X. Tan, H. Mao, J. Withers, G. Ben-Nissan, T.R. Hinds, Y. Kobayashi, F.F. Hsu, M. Sharon, J. Browse, S.Y. He, J. Rizo, G.A. Howe, N. Zheng, Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor, Nature 468 (2010) 400–405.
[55] E.K. Mibei, W.O. Owino, J. Ambuko, J.J. Giovannoni, A.N. Onyango, Metabolomic analyses to evaluate the effect of drought stress on selected African Eggplant accessions, J. Sci. Food Agric. 98 (2018) 205–216.
[56] M.Z. Mutwakil, N.H. Hajrah, A. Atef, S. Edris, M.J. Sabir, A.K. Al-Ghamdi, M. Sabir, C. Nelson, R.M. Makki, H.M. Ali, F.M. El-Domyati, A.S.M. Al-Hajar, Y. Gloaguen, H.S. Al-Zahrani, J.S.M. Sabir, R.K. Jansen, A. Bahieldin, N. Hall, Transcriptomic and metabolic responses of *Calotropis procera* to salt and drought stress, BMC Plant Biol. 17 (2017) 231.
[57] J.H. Widodo, E. Patterson, M. Newbigin, A. Tester, U. Roessner Bacic, Metabolic responses to salt stress of barley (*Hordeum vulgare* L.) cultivars, Sahara and Clipper, which differ in salinity tolerance, J. Exp. Bot. 60 (2009) 4089–4103.
[58] L.V. Dubovskaya, Y.S. Bakakina, E.V. Kolesneva, D.L. Sodel, M.R. McAinsh, A.M. Hetherington, I.D. Volotovski, cGMP-dependent ABA-induced stomatal closure in the ABA-insensitive Arabidopsis mutant abi1-1, New Phytol. 191 (2011) 57–69.
[59] N.A. Dar, I. Amin, W. Wani, S.A. Wani, A.B. Shikari, S.H. Wani, K.Z. Masoodi, Abscisic acid: a key regulator of abiotic stress tolerance in plants, Plant Gene 11 (2017) 106–111.
[60] H. Kikuzaki, M. Hisamoto, K. Hirose, K. Akiyama, H. Taniguchi, Antioxidant properties of ferulic acid and its related compounds, J. Agric. Food Chem. 50 (2002) 2161–2168.
[61] Z. Li, J. Yu, Y. Peng, B. Huang, Metabolic pathways regulated by abscisic acid, salicylic acid and gamma-aminobutyric acid in association with improved drought tolerance in creeping bentgrass (*Agrostis stolonifera*), Physiol. Plant. 159 (2017) 42–58.
[62] A. Borges, A.C. Abreu, C. Ferreira, M.J. Saavedra, L.C. Simoes, M. Simoes, Antibacterial activity and mode of action of selected glucosinolate hydrolysis products against bacterial pathogens, J. Food Sci. Technol. 52 (2015) 4737–4748.